Further Fractionations of Cigarette Smoke Condensate for Bioassays

Weak Acid and Neutral Subfractions and Combinations of Active Fractions*

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INTRODUCTION

Our joint effort to relate the tumorigenicity of cigarette smoke condensate (CSC) for mouseback skin to that of its active components is continuing (1). Reports describing the large-scale fractionation of CSC (1), subfractionation of the neutral part by chromatography on silicic acid (2) and the initial results of bioassay (3, 4) have appeared. This paper reports the methods used and yields obtained in further fractionations of the ether-soluble weak acid (WA$_E$) and neutral fractions. As in the former studies, the procedures used were designed to yield adequate amounts for bioassay of all fractions.

In a former study (3) evidence was obtained for the activity of weakly acidic material extracted by diethyl ether from alkaline aqueous extracts of CSC after removal of neutral and basic components and acidification to a pH of about 6.1 (WA$_E$). The simple phenols of CSC, which have been implicated in its tumorigenicity for mouseback skin (5, 6), would be expected to appear in this fraction. We decided to separate the volatile phenols (along with other volatile substances) from the nonvolatile components and to submit the resulting subfractions to bioassay. The present study reports details of their preparation; the results of bioassay have been reported (7).

Use of silicic acid chromatography for subfractionation of the neutral fraction of CSC resulted in incomplete recovery, as shown by chemical investigations (8) and by failure to recover all of the tumorigenic activity in the recombined subfractions (4). For this reason we decided to separate the neutral fraction into subfractions using solvent partitioning alone. The material soluble in aqueous methanol ("methanol-soluble neutrals", MSN) was partitioned between nitromethane and carbon disulfide. The material soluble in cyclohexane ("methanol-insoluble neutrals", MIN) was subjected to a 200-tube countercurrent distribution using cyclohexane and nitromethane phases. Results of bioassay using these MSN and MIN subfractions have been reported (10).

In an earlier study it was reported (4) that the neutral material eluted from silicic acid columns by methanol and passing into the aqueous methanol layer upon subsequent partition between this solvent and petroleum ether (MM$_W$) was one of four active neutral subfractions. Preliminary studies showed that MM$_W$ could be separated roughly according to molecular size using gel filtration (9). We decided to subject it to this procedure and submit the resulting subfractions for bioassay. At the same time we obtained the polynuclear aromatic hydrocarbon (PAH) containing neutral fractions from the silicic acid columns and the weak acids soluble in ether (WA$_E$) as in previous studies (1, 2). Thus we had available all three groups of substances previously shown to be active. These were submitted for bioassay in all possible combinations. The bioassay results are nearing completion and will be reported soon.

MATERIALS AND METHODS

Cigarette smoke condensate (CSC) was prepared in 1.0 kg batches at the Roswell Park Memorial Institute and shipped to Philadelphia or Athens under conditions previously described (1). The ether-soluble weak acid fraction (WA$_E$) and the two neutral subfractions (MSN, MIN) were prepared exactly as described in our first separation scheme (1). MM$_W$ and the other neutral fractions obtained by chromatography on silicic acid were prepared exactly as in our second scheme (2).

For the subfractionation of WA$_E$ a portion (53 %) was removed for control bioassay (F 27, Table 1, identical with F 8) and the remainder was subjected to steam distillation after removal of solvent. The tarry material

Table 1. Average yields of ether-soluble weak acid fractions (WA$_E$)*.

Fraction no.	Name	Used %	Range, gm min.	Range, gm max.	S. D.	Mean gm	S. E.
—	CSC	100	958	1067	19.9	1001.6	4.4
8	WA$_E$ for steam dist'n	47	38.4	56.7	5.1	46.8	1.1
27	WA$_E$, control bioassay	53	41.3	63.5	5.9	52.5	1.3
8+27	Total WA$_E$ gm/kg CSC	100	79.7	120.2	9.7	99.3	2.2
28	Distillation residue	35.25	21.1	39.9	4.5	28.7	1.0
29	Steam distillate	35.25	3.0	7.0	1.1	4.9	0.3
30	Reconstituted (28+29)	11.75	7.7	11.5	3.2	10.8	0.7

* From 20 runs using approximately 1 kg CSC (cigarette smoke condensate) per run. (See Table 3 for additional fractions from 9 of these runs.)

was covered with water in a long-necked 1000 ml round-bottom distillation flask equipped with a steam inlet tube dipping below the surface. While heating and magnetically stirring the contents, steam was admitted from a second flask containing boiling distilled water until about 1500 ml of condensate had collected. The two aqueous solutions were then saturated with sodium chloride at room temperature and the organic materials were extracted by shaking with diethyl ether. In the case of the residue a little acetone was required for complete solution. After drying over anhydrous sodium sulfate or magnesium sulfate a portion (25%) of each ethereal solution was removed for the reconstituted sample. Aliquots of the distillate fraction were removed for colorimetric and glc analyses for phenols. Solvent was removed from the remainders of the distillate and residue fractions and from the recombined fraction under previously described conditions (1). These three samples and the control sample were sealed under nitrogen in glass-stoppered flasks and shipped in Dry Ice for biological testing. See Table 1.

Total phenols were determined by the colorimetric method of *Smith* and *King* (11). For the glc analysis a 5 ml aliquot of the ether extract of the steam distillate was evaporated to dryness at room temperature under a current of nitrogen. The residue was taken up in methanol and brought to a volume of 0.2 ml. Standards of the individual phenolic compounds were made in methanol at concentrations such that a 10 microliter volume of suitable dilution would give a peak closely matching in height the corresponding peak from a 10 microliter volume of the sample solution when injected into the chromatograph[+]. Triplicate runs were made and average peak heights were compared with heights given by the knowns. Results were calculated to mg per kg of CSC, recorded in Table 2.

For subfractionation of the "methanol-soluble neutrals" (MSN), partitioning between carbon disulfide and nitromethane was shown in preliminary studies (9) to produce adequate separation. After removal of a portion (41.2%) for control bioassay (F 39, Table 3, identical with F 12 of our first scheme), solvent was removed from the remainder of the 80% aqueous methanol solution and the residue was partitioned between CS$_2$ and nitromethane, cross-extracting until separation was complete. This required a total of about 500 ml of nitromethane and 250 ml of CS$_2$ (caution: vapors explosive and toxic). A portion (40%) of each solution was removed for a reconstituted sample, and solvent was removed from these three and from the control sample before shipment in Dry Ice for bioassay. Results appear in Table 3.

Table 2. Phenols in steam distillate of fraction WA$_E$*.

Phenol	n**	Range, mg/kg CSC min.	Range, mg/kg CSC max.	S. D.	Mean mg/kg CSC	S. E.
Total, colorimetric	16	1730	3480	493.9	2623.8	123.5
GLC:						
Phenol	20	672	1630	209.5	1158.8	46.9
o-Cresol	20	117	397	75.3	246.5	16.8
m- + p-Cresols	20	430	943	125.2	641.6	28.0
2:4-Xylenol	19	53	214	43.6	119.8	10.0
3:5-Xylenol	19	87	355	79.6	226.1	18.3
Guaiacol	19	110	265	44.5	194.1	10.2
Sum of glc phenols	19	1945	3354	444.6	2597.6	102.0

* Ether-soluble weak acids of cigarette smoke condensate (CSC) using 5 ml aliquots of the ether solution of Fraction 29, Table 1.

** Number of runs used in calculating mean. Data for total phenols, guaiacol and the xylenols not available for all runs.

[+] Hewlett-Packard Model 5750 with thermal conductivity detection, Column 8 ft. × ¼ inch packed with H-P silicone phase UC-W98, 20% on acid-washed Chromosorb W, 60—80 mesh. Mention of specific commercial products does not constitute endorsement by the U.S. Department of Agriculture.

Table 3. Average yields of neutral subfractions prepared by solvent partition*.

No.	Fraction Name	Used, %	Range, gm min.	Range, gm max.	S. D.	Mean, gm	S. E.
—	CSC	100	993	1067	23.5	1005.7	7.8
	"Methanol-Insoluble neutrals" (MIN):						
32	MIN, control bioassay	41.2	55.3	137.7	28.3	93.6	9.4
—	MIN for ccd partition	58.8	79.8	206.2	44.3	137.8	14.8
—	Total MIN (sum)	100	135.1	343.9	72.5	231.4	24.2
33	Reconstituted MIN	23.52[a]	28.6	82.4	18.9	54.0	6.3
34	ccd tubes No. 0– 20	35.28[b]	0.7	1.7	0.3	1.1	0.1
35	ccd tubes No. 21– 45	35.28	2.3	3.6	0.6	3.0	0.2
36	ccd tubes No. 46–140	35.28	9.0	14.4	1.8	11.9	0.6
37	ccd tubes No. 141–165	35.28	4.5	24.1	6.5	10.0	2.2
38	ccd tubes No. 166–199	35.28	25.8	97.4	23.3	49.7	7.8
—	Total ccd fractions (sum of F 33–38)	58.8	75.7	189.3	39.5	129.7	13.2
	"Methanol-soluble neutrals" (MSN):						
39	MSN, control bioassay	41.2	16.3	22.0	1.8	19.2	0.6
—	MSN for partition	58.8	23.7	34.1	3.2	28.8	1.1
—	Total MSN (sum)	100	40.0	53.6	4.4	48.0	1.5
40	Reconstituted MSN	23.52[a]	8.4	12.9	1.4	11.2	0.5
41	Nitromethane-soluble MSN	35.28[b]	11.1	16.6	2.1	13.4	0.7
42	CS_2-soluble MSN	35.28	2.0	10.2	2.3	4.7	0.8
—	Total MSN fractions (sum of F 40–42)	58.8	23.7	34.4	3.5	29.3	1.2
—	**Total neutrals** before partition, gm/kg CSC (sum of total MIN plus total MSN)	100	187.2	397.5	72.9	279.4	24.3

* From 9 runs using approximately 1 kg of cigarette smoke condensate (CSC) per run. (At the same time as 9 of the 20 WA_E runs of Table 1.)

a) 0.4×58.8

b) 0.6×58.8. For reconstituted MIN and MSN, 40 % of each fraction was removed before evaporating solvent from the remainder.

The "methanol-insoluble neutrals" (MIN) consist of the cyclohexane-soluble material obtained in partitioning of the total neutrals between this solvent and 80 % aqueous methanol in our first scheme (1). After removal of a portion (41.2 %) of the cyclohexane solution for control bioassay (F 32, Table 3), solvent was removed from the remainder. The residue was added to the first five tubes of a 200-tube ccd machine in a mixture of nitromethane and cyclohexane using 40 ml of each solvent per tube. After the separation was complete, all of the material in both layers of pooled groups of tubes 0—20, 21—45, 46—140, 141—165 and 166—199 was recovered, first removing a portion (40%) for a reconstituted sample. These six and the control sample, after removal of solvent, were shipped in Dry Ice for bioassay. Results are recorded in Table 3.

The neutrals eluted from silicic acid by methanol were recovered by evaporation and were partitioned between aqueous methanol and petroleum ether to obtain MM_W exactly as in our second fractionation scheme (2). Four portions of 15 % each were removed for control bioassay and for combination with WA_E (F 50), the PAH-containing neutral fractions (F 52), and both of these (F 53). Solvent was removed from the remainder (40 %)

and the residue was added to columns of Bio-Beads* S—X2, 200—400 mesh, each 200 cm in length×2.5 cm diameter, using 165 gm of Bio-Beads and 1.0 gm of sample per column, and benzene as eluant. The eluates were collected in five fractions of 110, 40, 60, 100 and 160 ml each from each column. These amounts had been shown in preliminary work (9) to give a rough separation into fractions with average molecular weights of 2020, 790, 450, 240 and less than 240. A portion (40 %) of each pooled fraction from the columns was removed for a reconstituted sample. These six and the control sample were shipped, after removal of solvent, in Dry Ice for bioassay. At the same time that the MM_W for this experiment was obtained by chromatography on silicic acid, WA_E and the neutral fractions from the column were also obtained. The PAH-containing neutral fractions (for F 51, 52 and 53, Table 5) were obtained as before (2) except that the material eluted by 25 % benzene in petroleum ether was not separated into two fractions (19 and 20) by partition between cyclohexane and DMSO. Instead, four portions of 15 % each were removed from this

* Bio-Rad Laboratories, Richmond, California, USA.

Table 4. Average yields of neutral subfractions prepared by gel filtration*.

No.	Fraction Name	Used, %	Range, gm min.	Range, gm max.	S. D.	Mean, gm	S. E.
—	CSC	100	991	1025	11.1	1007.9	2.3
1	CSC for control bioassay	6.1[a]	—	—	—	61.0	—
—	CSC for fractionation	93.9	929.8	964	11.1	946.9	2.3
15	Neutrals, control bioassay	13.0	12.4	41.1	6.1	27.6	1.3
—	N for silicic acid chrom.	87.0	116.5	269.9	28.4	195.7	5.8
—	Total neutrals	100	131.3	311.0	32.8	223.3[b]	6.7
26	MM_W for control bioassay	13.05[c]	1.9	5.1	0.8	2.9	0.2
—	MM_W for gel filtration	34.80[d]	5.5	11.8	1.8	7.6	0.4
—	Total MM_W	87	—	—	—	19.2[e]	—
44	Gel fraction No. 1 (110 ml)	26.1[f]	0.8	3.9	0.7	1.6	0.1
45	Gel fraction No. 2 (40 ml)	26.1	0.2	0.8	0.1	0.4	0.03
46	Gel fraction No. 3 (60 ml)	26.1	0.4	1.5	0.3	0.8	0.06
47	Gel fraction No. 4 (100 ml)	26.1	1.2	4.7	0.9	2.3	0.17
48	Gel fraction No. 5 (160 ml)	26.1	0.5	2.6	0.5	1.3	0.11
49	Reconstituted gel fractions	8.7[g]	1.3	4.7	0.9	2.2	0.18

* From 24 runs of approximately 1 kg of cigarette smoke condensate (CSC) each run. See Table 5 for additional fractions from these same runs.
a) 61.0 gm was removed from each kg of CSC for fraction 1.
b) From 946.9 gm CSC average. Equivalent to 235.9 gm neutrals per kg CSC.
c) 0.87×15. MM_W is the methanol eluate, aqueous methanol-soluble part. See Table 5 for three more such portions used in fractions 50, 52 and 53.
d) 0.87×40. Each gel column 200×2.5 cm used 165 gm Bio-Beads S–X2 (Bio-Rad Labs., Richmond, Calif.), 200–400 mesh, 1.0 gm sample, benzene eluant.
e) 4×2.9 plus 7.6 gm. From 946.9×0.87 gm CSC. Equivalent to 23.3 gm MM_W per kg CSC.
f) 0.75×34.8. 110, 40, 60, 100 or 160 ml eluate from each column pooled.
g) 0.25×34.8. For the reconstituted fraction, 25 % of each pooled eluate was removed before evaporating solvent from the remainder.

Table 5. Average yields of fractions of CSC used in combination*.

No.	Fraction Name	Used, %	Range, gm min.	Range, gm max.	S. D.	Mean, gm	S. E.
8[a]	WA_E for control bioassay	13.0	7.2	16.3	1.7	11.5	0.4
—	WA_E excess	48.0[b]	22.0	70.1	10.8	42.6[c]	2.3
19+20	N_{BPE} excess	34.8[d]	6.0	20.8	3.3	9.8[c]	0.7
22	N_{B-DMSO} excess	34.8[d]	1.8	5.8	1.2	3.3[c]	0.3
26	MM_W, control bioassay	13.05[e]	1.9	5.1	0.8	2.9	0.2
43	"PAH", control bioassay	13.05	2.6	7.9	1.3	4.5	0.3
50	WA_E and MM_W combined	13.0	10.0	19.2	2.0	14.3	0.4
51	WA_E and "PAH" combined	13.0	10.1	26.7	3.2	15.7[f]	0.7
52	MM_W and "PAH" combined	13.0	4.8	11.8	1.9	7.7	0.4
53	WA_E, MM_W and "PAH"	13.0	13.5	23.5	2.7	18.7	0.5

* From the same 24 runs used for the fractions of Table 4.
a) Numbering of fractions 1–26 in Tables 4 and 5 is the same as in earlier publications (1,2).
b) WA_E is the ether-soluble weak acid fraction. Remainder after removal of four portions of 13 % each for fractions 8, 50, 51 and 53.
c) Average of 22 runs.
d) 0.87×40. Remainder after removal of four portions of 15 % each for fractions 43, 51, 52 and 53. F 19+20 (N_{BPE}) is the neutral fraction eluted from silicic acid by 25 % benzene in petroleum ether. F 22 (N_{B-DMSO}) is that part of the benzene eluate soluble in dimethyl sulfoxide. These fractions were shown in an earlier publication (2) to contain most of the polynuclear aromatic hydrocarbons (PAH) of CSC.
e) 0.87×15. MM_W is that part of the methanol eluate soluble in aqueous methanol.
f) Average of 23 runs.

eluate. The material eluted by benzene was separated by partition between cyclohexane and dimethyl sulfoxide (DMSO) as before (2) to obtain F 22, the part soluble in DMSO. Four portions of F 22 of 15 % each were removed. One portion of F 22 was combined with F 19–20 to give F 43, the PAH-containing neutral fractions, for bioassay. One portion of each (F 19–20, F 22) was combined with MM_W for F 52. One portion of each was combined with a 13 % portion of the ether-soluble weak acid fraction (WA_E), obtained exactly as before (1) for F 51, and the last was com-

bined with both of these for F 53. In addition, a 13 % portion of WA_E was used for a control bioassay sample, and a control CSC sample of 61 gm was removed from each kg before fractionation. In all, 24 kg were fractionated. Results are found in Tables 4 and 5.

RESULTS AND DISCUSSION

In earlier publications (1, 2) we outlined as our objective the preparation of fractions in such a way that all fractions obtained in any one fractionation scheme

could be tested, and we proposed to test them in the relative proportions in which they were obtained from CSC. In this way we hoped that the activity could be reproduced by a combination of active fractions, discarding the bulk of the condensate only after proving it inactive. We have now reached the point where we can begin to attempt this, as indicated in Table 5. The biological results have shown that there are only three groups of substances that contribute importantly to the tumorigenic activity of CSC: the ether-soluble weak acids (WA_E), the PAH-containing neutral fractions, and a neutral fraction (MM_W) eluted from silicic acid by methanol and passing into the aqueous methanol layer upon subsequent partition between this solvent and petroleum ether (3, 4). These three groups have been recombined in all possible ways in the four mixtures of Table 5. Control tests are also being made on the three individual groups (Table 5) and on the CSC from which they were prepared (Table 4). In future work we hope to obtain and test highly purified subfractions of PAH, MM_W and WA_E, separated from the inactive materials these fractions now contain. Thus we hope to be able to reproduce the tumorigenic action of CSC for mouseback skin with, eventually, a mixture of known compounds.

Regarding the nature and identity of the active materials in these groups of fractions, the PAH-containing neutral fractions have now been shown to be tumor initiators, as would be expected, but MM_W is a promoter only as is WA_E (10). As the biological results using the fractions of Tables 1 and 2 showed (7), the promoting activity of WA_E resides not in the volatile phenols it contains, but in the nonvolatile residue from steam distillation (F 28 of Table 1). This fraction has been shown to contain a mixture of fatty acids which could account for its promoting activity (9). Bioassay using a synthetic mixture of the known fatty acids in the proportions in which they occur in WA_E is currently under way at Buffalo (7). The biological results using the PAH-containing neutral subfractions (MIN of Table 3) indicate that there are at least two, but probably many more, constituents contributing to this activity, on the basis of partition coefficients (10). The identity of the promoters of MM_W is completely unknown, but use of the subfractions obtained by gel filtration (Table 4), currently under test, will hopefully simplify the task of identification.

Questions of possible identities between various fractions, as for example the active constituents of the MSN of Table 3 and the MM_W of Table 4, or those of the MIN of Table 3 and the PAH-containing fractions (19, 20 and 22) of Table 4, cannot be settled until these constituents have been isolated and identified. Chemical investigations of these fractions are currently being continued at the Athens laboratory. Also, in order to determine whether the fatty acids of WA_E are the only active constituents, they must be separated from it so that the remaining material can be tested biologically. This is currently being attempted.

Variability in yields of some of the fractions is quite high. Consequently, mean yield of a fraction should not be taken as a precise measure of the content of any substance in CSC. Rather, the S.D., range and S.E. of the mean are indications of the variability on repeated fractionation of separate kg of CSC, individually prepared as needed, from commercial cigarettes, over a period of a year or more, with several different persons performing the various operations involved in the fractionations at different times. Viewed in this light it is perhaps remarkable that variability was not greater.

Mean yields of the phenols in the steam distillate are lower than have been reported for the phenols in cigarette smoke (12, 13). Two factors may account for this: the scale on which WA_E was prepared in our work was thousands of times greater than in the analytical studies referred to; and the butt length to which the cigarettes were smoked (not less than 20 mm in our work) may have been greater than in some of the analytical studies.

Total yields (sum) of the fractions prepared from MM_W by gel filtration averaged greater than 100% (112.8). This was not true in the preliminary study (9), in which an average yield of 84% was reported. The reason for this is not clear. Traces of solvent may have remained, but all fractions were evaporated to constant weight at reduced pressure in a rotating still. There is no indication that the gel is soluble to any extent in benzene; tests with the S-X2 gel showed no residue. In any case, the columns were thoroughly washed with benzene before loading. Actually, the columns retained an undetermined but small amount of sample, as indicated by color remaining after elution. These are matters for further investigation.

SUMMARY

Cigarette smoke condensate (CSC) was fractionated for biological experiments examining tumorigenic activity on mouse skin. The active fractions were combined in various ways in order to determine their additive effects. Steam distillation of the ether-soluble weak acid fraction (WA_E) provided two subfractions which were submitted for assay separately and in combination. The total neutral fraction was separated by solvent partition between cyclohexane and 80% aqueous methanol as in a previous study and each subfraction was further fractionated: the part insoluble in aqueous methanol (MIN) was subjected to a 200-tube countercurrent distribution between cyclohexane and nitromethane to provide five subfractions; and the part soluble in aqueous methanol (MSN) was separated into two subfractions by partition between nitromethane and carbon disulfide. Reconstituted MIN and MSN fractions were prepared by recombination of their subfractions.

Finally, the total neutrals were separated into five fractions by chromatography on silicic acid as done in a previous study in order to obtain the active fraction

eluted by methanol and soluble in aqueous methanol (MM$_W$). This was subjected to gel filtration using benzene as eluant. The five subfractions of MM$_W$ were submitted for assay separately and in combination. In addition to these fractions, four combinations of active fractions were prepared: WA$_E$ with MM$_W$; WA$_E$ with the polynuclear aromatic hydrocarbon (PAH) containing neutral fractions; MM$_W$ with the PAH-containing neutrals, i. e. all of the active neutral fractions; and WA$_E$ with MM$_W$ and the PAH-containing neutrals. The last comprises essentially all of the fractions shown to possess tumorigenic activity for mouseback skin in previous work.

ZUSAMMENFASSUNG

Für Untersuchungen auf dem Gebiet der experimentellen Tumorforschung wurden Cigarettenrauchkondensate (CSC) fraktioniert, und die wirksamen Fraktionen wurden zur Bestimmung ihrer additiven Wirkungen in verschiedenartiger Weise kombiniert. Die Wasserdampf-destillation der ätherlöslichen, schwach sauren Fraktion (WA$_E$) ergab zwei Unterfraktionen, die einzeln und in Kombination in diesen Tierexperimenten untersucht wurden. Die neutrale Gesamtfraktion wurde wie in einem früheren Versuch durch Lösungsmittelverteilung zwischen Cyclohexan und 80%igem wässerigen Methanol aufgetrennt, und jede Unterfraktion wurde weiter fraktioniert. Der in wässerigem Methanol unlösliche Anteil (MIN) wurde einer 200stufigen Gegenstromver-teilung zwischen Cyclohexan und Nitromethan unter-worfen, was fünf Teilfraktionen ergab. Der in wässe-rigem Methanol lösliche Anteil (MSN) wurde durch Ver-teilung zwischen Nitromethan und Schwefelkohlenstoff in zwei Unterfraktionen aufgeteilt. Die MIN- und MSN-Fraktionen wurden für den Versuch durch Ver-einigung ihrer Unterfraktionen rekombiniert.

Schließlich wurde die gesamte Neutralfraktion wie in einer früheren Untersuchung durch Chromatographie auf Kieselgel in fünf Teile fraktioniert, damit die aktive Fraktion erhalten wurde, die durch Methanol eluiert wird und in wässerigem Methanol löslich ist (MM$_W$). Diese aktive Fraktion wurde mit Benzol als Eluierungs-mittel einer Gel-Filtration unterworfen. Die fünf Unter-fraktionen von MM$_W$ wurden einzeln und kombiniert auf ihre carcinogene Wirkung untersucht. Neben diesen Fraktionen wurden die aktiven Fraktionen viermal fol-gendermaßen kombiniert und die sich ergebenden Ver-bindungen wiederum untersucht: WA$_E$ zusammen mit MM$_W$; WA$_E$ zusammen mit den neutralen Fraktionen, in denen die polynuklearen aromatischen Kohlenwasser-stoffe (PAH) enthalten sind; MM$_W$ zusammen mit PAH enthaltenden neutralen Fraktionen, d. h. alle aktiven neutralen Fraktionen; und schließlich WA$_E$ zu-sammen mit MM$_W$ und den PAH enthaltenden Neutral-fraktionen. Die letztgenannte Kombination umfaßt im wesentlichen alle Fraktionen, die sich in früheren Ex-perimenten auf der Rückenhaut der Maus als carcinogen erwiesen haben.

RESUME

Le condensat de fumées de cigarettes (CSC) a é fractionné pour permettre des expériences biologiqu concernant l'activité carcinogène sur la peau de sour Les fractions actives ont été combinées de différent manières afin de déterminer leurs effets additifs. distillation à la vapeur de la partie »acide faible solub à l'éther« (WA$_E$) produit deux sous-fractions, do l'activité carcinogène a été testée séparément et e combinaison. On a séparé la fraction neutre totale pa partition en solvant, en utilisant du cyclohexane et un solution aqueuse de méthanol à 80%, comme dan des travaux antérieurs, et l'on a refractionné chaqu sous-fraction: La partie insoluble dans le méthano aqueux (MIN) a été séparée en cinq sous-fraction dans un distributeur à contre-courant de deux cent tubes, et utilisant du cyclohexane et du nitrométhane La partie soluble dans le méthanol aqueux (MSN) été séparée en deux sous-fractions par partition entre le nitrométhane et le disulfure de carbone. Des fractions MIN et MSN reconstituées ont été préparées par recombinaison de leurs sous-fractions.

Finalement la fraction neutre totale a été séparée en cinq sous-fractions par chromatographie sur colonne de silice (comme dans une étude précédente) pour obtenir la fraction active éluée par le méthanol aqueux (MM$_W$). On a filtré cette fraction sur gel, en éluant avec du benzène. Les cinq sous-fractions de MM$_W$ ont été testées pour leur activité carcinogène séparément et en combinaisons. En plus, quatre combinaisons de fractions actives ont été préparées: WA$_E$ + MM$_W$; WA$_E$ avec les fractions neutres contenant les hydro-carbures aromatiques polynucléaires (PAH); MM$_W$ + les fractions neutres contenant des PAH, c. à. d. toutes les fractions neutres actives; WA$_E$ + MM$_W$ + les fractions neutres contenant les PAH. Cette dernière com-binaison contient en particulier toutes les fractions connues comme ayant une activité tumorigénique sur la peau postérieure de souris d'après des travaux antérieurs.

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